PB85-243129

# Interactions of Chlorine-Produced Oxidants (CPO) and Salinity in Affecting Lethal and Sublethal Effects in the Eastern or American Oyster, ´Crassostrea virginica´ (GMELIN), Infected with the Protistan Parasite 'Perkinsus marinus'

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

Aug 85

U.S. Department of Commerce
National Technical Information Service
NTIS.

PB85-243129

EPA/600/D-85/196
August 1985

# Interactions of Chlorine-Produced Oxidants (CPO) and Salinity in Affecting Lethal and Sublethal Effects in the Eastern or American Oyster, *Crassostrea virginica* (GMELIN), Infected with the Protistan Parasite, *Perkinsus marinus*

by

Geoffery I. Scott, Douglas P. Middaugh, and Tommy I. Sammons
Environmental Research Laboratory
Gulf Breeze, FL 32561

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Gulf Breeze, FL 32516

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

**TECHNICAL REPORT DATA**
*(Please read Instructions on the reverse before completing)*

| | |
|---|---|
| 1. REPORT NO. EPA/600/D-85/196 | 2. |
| 3. RECIPIENT'S ACCESSION NO. PB85 243129 7AS | |
| 4. TITLE AND SUBTITLE INTERACTIONS OF CHLORINE-PRODUCED OXIDANTS (CPO) AND SALINITY IN AFFECTING LETHAN AND SUBLETHAL EFFECTS IN THE EASTERN OR AMERICAN OYSTER, Crassostrea virginica (GMELIN), INFECTED WITH THE PROTISTAN PARASITE, Perkinsus marinus | 5. REPORT DATE August 1985 |
| | 6. PERFORMING ORGANIZATION CODE |
| 7. AUTHOR(S) G. I.Scott, D. P. Middaugh, and T. I. Sammons | 8. PERFORMING ORGANIZATION REPORT NO. |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS | 10. PROGRAM ELEMENT NO. |
| | 11. CONTRACT/GRANT NO. |
| 12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Gulf Breeze, FL 32516 | 13. TYPE OF REPORT AND PERIOD COVERED |
| | 14. SPONSORING AGENCY CODE EPA/600/04 |

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Exposure of oysters, infected with the protistan parasite, Perkinsus marinus, to high salinity and high salinity, chlorine-produced oxidants (CPO) conditions was quite toxic. The combination of high salinity and CPO exposure generally increased toxicity above levels measured in high salinity controls and was generally correlated with parasitism by the protistan parasite, P. marinus. Low salinity and the combination of low salinity and CPO exposure resulted in a significant reduction in P. marinus related oyster mortalities. Low salinity, CPO exposure reduced oyster mortalities from P. marinus infections by as much as 56%. The observed lower mortality rates in oysters exposed to low salinity, glycogen and free amino acid levels, which may have limited the intensification of the disease to lethal hypnospore infection levels.

17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| | | |

| | | |
|---|---|---|
| 18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC | 19. SECURITY CLASS *(This Report)* UNCLASSIFIED | 21. NO. OF PAGES 28 |
| | 20. SECURITY CLASS *(This page)* UNCLASSIFIED | 22. PRICE |

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

# INTERACTIONS OF CHLORINE-PRODUCED OXIDANTS (CPO) AND SALINTY IN AFFECTING LETHAL AND SUBLETHAL EFFECTS IN THE EASTERN OR AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN), INFECTED WITH THE PROTISTAN PARASITE, *PERKINSUS MARINUS*

Geoffery I. Scott, Douglas P. Middaugh, and Tommy I. Sammons

SUMMARY

Exposure of oysters, infected with the protistan parasite, Perkinsus marinus, to high salinity and high salinity, chlorine-produced oxidants (CPO) conditions was quite toxic. The combination of high salinity and CPO exposure generally increased toxicity above levels measured in high salinity controls and was generally correlated with parasitism by the protistan parasite, P. marinus.

Low salinity and the combination of low salinity and CPO exposure resulted in a significant reduction in P. marinus related oyster mortalities.

Low salinity, CPO exposure reduced oyster mortalities from P. marinus infections by as much as 56%. The observed lower mortality rates in oysters exposed to low salinity, CPO regimes appeared to be related to reductions in tissue glycogen and free amino acid levels, which may have limited the intensification of the disease to lethal hypnospore infection levels. Earlier studies by several authors (Lange, 1970; Block et al., 1977; Soniat & Koenig, 1982) have indicated that exposure to low salinity or CPO reduces free amino acid levels in oysters and that free amino acid levels decrease with increased parasitism by P. marinus. Results from this study indicate that the combined effects of low

salinity and CPO exposure lower mortality resulting from P. marinus infections perhaps due to the reduction of free amino acid levels in adult oysters. Reductions in free amino acid levels would prevent and limit the spread and intensification of the disease, thus increasing oyster survival.

Low salinity alone reduced oyster mortalities from P. marinus infection, but only by 25%. Reduced free amino acid levels were probably responsible for limiting mortality from P. marinus due to a delay and limitation of infection to lethal levels.

## INTRODUCTION

Chlorine has been widely used to disinfect sewage plant effluents and potatable drinking water supplies and to control antifouling organisms at power plants (White, 1972; Hall et al., 1981). Typically, sewage waste waters are chlorinated at a specified level (i.e., 2 mg/L chlorine residual) for specified contact time (i.e., 30 min) to lower coliform bacterial densities to levels safe for discharge in aquatic environments. Discharges into estuarine habitats of sewage effluent containing excess chlorine concentrations may be potentially toxic to fish and invertebrates, particularly the eastern or American oyster, Crassostrea virginica (Gmelin) (Scott, 1983).

Chlorinated discharges may be acutely toxic to oyster larvae (Rosenberg et al., 1980; Roberts & Casey, in press). Roberts and Casey (in press) reported a 48-h median lethal concentration (LC50) of 23 µg/L chlorine-produced oxidant (CPO) concentration for oyster embryos exposed from fertilization to straight-hinge veliger stage. Rosenberg et al. (1980) reported a 96-h LC50 for post straight-hinge oyster larvae of 300 µg/L CPO and suggested that the 96-h LC50 of recently set spat was greater than 500 µg/L CPO.

Chlorinated effluents may be chronically toxic to adult oysters, causing significant mortality at CPO concentrations of 660 µg/L and significant, irreversible metabolic effects (decreased physiological performance) at CPO concentrations as low as 110 µg/L (Scott, 1979). Additionally, the sensitivity of oysters to chlorination exposure varies significantly from season to season, with adults being three to four times more sensitive to exposure during the summer (postspawning) than spring (prespawning). When oysters were compared under identical temperature, salinity, and CPO-exposure regimes, Scott et al. (1980) reported greater sublethal sensitivity in summer oysters as respiration rates

were significantly higher in the summer than the spring. Both spring and summer groups were infected with the protistan parasite, Perkinsus marinus.

Studies on the epizootiology (the abrupt increase of a disease in animals other than humans) of P. marinus have indicated that this parasite is basically a summer disease, causing peak mortalities (>50%) in August and September when water temperatures and salinity values are maximal (Mackin 1962; Quick and Mackin, 1971). Temperatures greater than 25°C are required for high infection rates to develop, while temperatures less than 10°C have been shown to inhibit hypnospore growth (Andrews and Hewatt, 1957; Perkins, 1966). Salinity has also been shown to be important, as infection intensities and associated mortalities are greatest in high-salinity waters (Mackin and Wray, 1952; Ray, 1954). Low salinity exposure tends to delay development of heavy infection intensities, to depress infection intensities and the incidence of infection, and to significantly enhance survival (Mackin & Wray, 1952; Ray et al., 1953; Ray, 1954; Andrews & Hewatt, 1957).

As earlier discussions indicated, oysters are much more susceptible to chlorination exposure during summer months. This increased summer susceptibility to chlorine exposure may result from lessened resistance in the oyster as a result of P. marinus infection. Several authors (Couch, 1978; Sinderman, 1978) have described the interactions between increased levels of pollution and increased incidences of parasitism and disease in marine organisms.

The purpose of this study was to investigate the effects of chlorination exposure on the American or eastern oyster, Crassostrea virginica (Gmelin), infected with the protistan parasite, Perkinsus marinus, so as to ascertain any potential interactions between toxicant and parasite in defining the lethal and sublethal response of the oyster to combined toxicant/parasite effect. All tests were conducted at high (25°/$_{oo}$) and low (<10°/$_{oo}$) salinities, due to known interactions of high salinity in increasing oyster mortalities from enhanced P. marinus infection intensities, and low salinity in decreasing oyster mortalities due to reduced P. marinus infection intensities.

## MATERIALS AND METHODS

### Collection and Acclimation

Adult oysters (6-12 cm in height) were collected from Leadenwah Creek (latitude N 32°36'; longitude W 80°15'), a

large tidal tributary of the North Edisto River Estuary in South Carolina, on 21 June 1982. Following collection, oysters were transported immediately to the laboratory where they were scrubbed and all fouling organisms removed. After numbering and measuring the height of individual oysters, 120 animals were placed in each test aquarium (circular tanks: 152.4-cm radius x 30-cm depth with a working volume of 310 L) and were acclimated to ambient light, salinity ($\bar{x}$ = 22.8°/$_{oo}$), and temperature ($\bar{x}$ = 30.1°C) for 39 d. Unfiltered sea water was pumped in daily from the Stono River at high tide. Tanks were cleaned daily during acclimation. An additional 24 d of acclimation beyond the scheduled 15-d period were required because high rainfall in the area depressed high-tide salinities to values below those necessary for the experiment.

Following acclimation, oysters in each tank were exposed for 60 d to one of the several treatments described in Table 1. Tests were conducted from August through October 1982.

## Exposure Conditions

Low salinity sea water (treatment E) was prepared by diluting incoming ambient sea water with groundwater from a shallow well. Normal ambient salinity at high tides ranged from 21 to 25°/$_{oo}$. Low salinity water of 8-10°/$_{oo}$ was desired, which required approximately a 40%:60% dilution of sea water:groundwater. Salinity averaged 9.2°/$_{oo}$ in the low salinity control sea water. Ambient temperatures of the resulting low salinity sea water ($\bar{x}$ = 23.9°C) were not significantly lower than ambient sea water temperatures ($\bar{x}$ = 24.6°C).

Preparations of chlorinated sea water were made at both low (8-10°/$_{oo}$, $\bar{x}$ = 9.3) and high (21-25°/$_{oo}$, $\bar{x}$ = 22.8) salinities by daily addition of NaOCl (Fisher Reagent Grade, 5%) to each tank at appropriate concentrations and dilutions. Nominal chlorine concentrations were 1.00, 0.56, and 0.10 mg/L in both high salinity (treatments B, C, and D, respectively) and low salinity (treatments F, G, and H, respectively) sea water. Exposure temperatures averaged 24.1°C in high salinity, CPO exposures compared to 23.6°C in low salinity, CPO exposures.

Oysters were also exposed to ambient high salinity (21-25°/$_{oo}$) sea water (treatment A), to serve as indicators of natural or control conditions.

A total of 120 oysters were placed in each test aquaria and were exposed to each treatment for 60 d (Table 1). Continuous aeration of each aquarium was provided by a piston pump (DP Model 440), while circulation was provided by a submersible pump (Little Giant) attached to a programmable timer to provide a realistic simulation of semidiurnal tidal currents in the Stono River estuary (5 h of flow followed by 1 h of slack water). Oysters fed on algae present in the sea water and were exposed to the ambient light:dark (14 h:10 h) cycle and sea water temperatures. Water changes were made and test aquaria were cleaned daily throughout the experiment.

## Test Parameters Measured

During each experiment, the following measurements were used to assess the physiological effects of each treatment on adult oysters:

1. Survival and mortality percentages were recorded daily using three replicates per treatment.
2. Prior to and at the end of each exposure, shell height (cm) was measured, using Vernier calipers.
3. Chlorine-produced oxidant (CPO) concentrations (free plus combined chlorine, bromine, and other residual oxidants) were measured with a Fisher Titrator (Model 397). Phenylarsine oxide and pH 4 buffer were standard Fisher reagents. The 5% KI solution and chlorine (Fisher NaOCl analytical reagent, minimum 5% NaOCl) stock solutions were prepared with deionized water. Stock solutions were kept in lightproof containers to prevent photodecomposition. Measurements of CPO concentrations employed procedures outlined in Standard Methods (APHA, 1971). All water samples were taken directly from treatment aquaria at least every 2-3 d during each experiment. Agitation of samples was avoided to reduce the potential for error by photodecomposition or flashing-off of CPO (EPA, 1974). The mixing order of Crecelius *et al*. (1978) was used in each titration: addition of 2 ml of pH 4 buffer and 2 ml of 5% KI solution to the sampling cup followed by addition of the sea water sample. Values are reported in mg/L.
4. In each experiment, ambient sea water temperature and salinity were measured daily in the incoming sea water from the Stono River. A mercury thermometer and a refractometer (American Optics) were used to measure temperature and salinity, respectively. In addition, periodic measurements of dissolved oxygen (Yellow Springs Instrument Company) and

Table 1. Description of treatment codes and physical/chemical parameters measured in each of the various salinity and chlorination treatments used in this study. Statistical measurement included calculation of mean ($\bar{x}$) and standard error ($s/\sqrt{n}$). Single (*) or double (**) asterisks indicate measured CPO concentrations in each low salinity, CPO treatment were significantly ($p<0.05$) or very significantly ($p<0.01$) different from similar high salinity, CPO treatments.

| Treatment Code | Treatment Description[1] | Measured CPO Concentration (mg/L)[2] | | Exposure Salinity (ppt)[3] | | Exposure Temperature (°C)[4] | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | $s/\sqrt{n}$ | $\bar{x}$ | $s/\sqrt{n}$ | $\bar{x}$ | $s/\sqrt{n}$ |
| A | High salinity control | 0.00 | 0.000 | 22.6 | 0.42 | 24.2 | 0.40 |
| B | High salinity, 1.00 mg/L NaOCl concentration | 0.06 | 0.007 | 22.9 | 0.21 | 24.2 | 0.40 |
| C | High salinity, 0.56 mg/L NaOCl concentration | 0.03 | 0.005 | 22.7 | 0.04 | 24.0 | 0.39 |
| D | High salinity, 0.10 mg/L NaOCl concentration | 0.01 | 0.003 | 22.9 | 0.21 | 24.0 | 0.39 |
| E | Low salinity control | 0.00 | 0.000 | 9.2 | 0.18 | 23.9 | 0.38 |
| F | Low salinity, 1.00 mg/L NaOCl concentration | 0.11** | 0.008 | 9.3 | 0.18 | 23.6 | 0.39 |
| G | Low salinity, 0.56 mg/L NaOCl concentration | 0.06** | 0.007 | 9.6 | 0.59 | 23.6 | 0.39 |
| H | Low salinity, 0.10 mg/L NaOCl concentration | 0.02* | 0.004 | 9.0 | 0.17 | 23.6 | 0.39 |

1 = NaOCl refers to nominal NaOCl concentrations added.
2 = Sample size (n) ranged from 36 to 38.
3 = n ranged from 57 to 58.
4 = n ranged from 51 to 52.

pH (Corning pH meter) were made. Dissolved oxygen measurements showed that oxygen concentrations were always at saturation. The pH ranged from 7.40 to 8.20 during the study.

5. Fecal production or biodeposition rate measurements were made every 2-3 d in all treatments by observing the number of oysters producing feces per day.

6. Perkinsus marinus densities in oyster tissues were determined using methods described by Ray (1952, 1966), Quick and Mackin (1971), and Farley (1965), but modified slightly. Fifteen adult oysters were removed from each test aquaria on days 0, 15, 30, and 60 of the experiment. A total of 490 oysters were examined for P. marinus densities. Each oyster was scrubbed thoroughly and then opened. The gill tissues and gonads were removed for tissue respiration and gonadal index measurements. The rectum and labial palps were removed, weighed, and then placed into culture tubes (17 x 125 mm with screw caps) containing 10 ml of fluid thioglycollate medium which had been fortified with myostatin (E.R. Squibb and Sons, Inc., NDC0003-0543-20 and M175C/59320) and chlormphenicol (Park-Davis M379). Inoculated test tubes were incubated in the dark at 25°C for a minimum of 7 d to a maximum of 18 d. Tissues were checked after 4, 10, and 18 d of incubation for the presence of hypnospores.

After incubation, each piece of tissue was removed from the culture media, blotted to remove excess liquid, and was placed on a labeled microscope slide. Several drops of Lugol's aqueous iodine potassium iodide solution was then added to stain each tissue. After 10 min, a cover glass was pressed onto the preparation, and examinations were then made at 50x and 100x magnifications using an Olympus light microscope. Tissue explant size and slide preparation thickness were kept as constant as possible to minimize error in infection density measurements.

Incidence of infection and infection density were determined by counting the number of hypnospores in either a 5-mm (50x) or 2-mm (100x) field of view. Three replicate counts of the most heavily infected area of each tissue were made to provide statistical quantification of infection density. Based upon the average infection density, oysters were then classed according to the method used by Ray (1953, 1954):

a. negative infection (code #0) with no hypnospores present;
b. Very light infection (1) with a density of 1-5 hypnospores/5-mm field;

c. Light infection (2) with a density of 6-10 hypnospores/5-mm field;
d. Lightly moderate infection (3) with a density of 11-30 hypnospores/ 5-mm field;
e. Moderate infection (4) with a density of 31-300 hypnospores/5-mm field;
f. Moderately heavy infection (5) with a density of 301-1000 hypnospores/5-mm field;
g. Heavy infection (6) with a density of 1001-3000 hypnospores/5-mm field; and
h. Very heavy infection (7) with a density of >3001 hypnospores/5-mm field.

Moderate (4) to very heavy (7) infection densities have generally been correlated with parasite infection densities which will cause immediate oyster death.

Infection density code numbers were averaged for each group of oysters to produce the weighted incidence of infection, which is a value reflecting both the proportion of the population infected and the intensity of infection. In addition, calculations of incidence of infection were made for each group of oysters from each of the various treatments.

## Statistical Treatment

Statistical analysis of each experimental measurement in oysters from each treatment included determination of mean, standard deviation, and standard error. T-tests (parametric) and Wilcoxon T-tests (nonparametric) were used to determine if measured differences between various treatments were statistically significant. The 95% confidence level ($p<0.05$) was the minimum statistical significance accepted.

# RESULTS AND THEIR INTERPRETATION

## Salinity Effects

Results indicating the effect of high and low salinity exposure on oyster mortalities associated with infections of *P. marinus* are depicted in Figure 1A. Oyster mortalities were virtually the same in high and low salinity during the first 33 d of exposure (16% vs. 18% mortality, respectively); however, after 60 d of exposure, oyster mortalities were significantly ($p<0.05$) reduced in the low salinity treatment by an average of 25% ($\bar{x}$ = 35%) when compared to high salinity controls ($\bar{x}$ = 46.7%).

Measurement of fecal production or biodeposition rates are listed in Table 2. Fecal production was significantly ($p \leq 0.01$) lower in low salinity controls ($\bar{x}$ = 64.7%) when compared to high salinity controls ($\bar{x}$ = 83.4%). The lower rate of fecal production in the low salinity oysters was probably related to the lower phytoplankton density (food availability) in the low salinity treatment.

Table 2. Summary of fecal production values measured in oysters exposed to various salinity/chlorination conditions. Note the significant ($p \leq 0.01$) reduction in fecal production in low salinity and low salinity, CPO-exposed oysters. Statistical parameters included calculations of mean ($\bar{x}$), standard error ($s/\sqrt{n}$), sample size (n), and Students t-value. Asterisks (**) indicate sample means were significantly ($p \leq 0.01$) different.

| Treatment | Nominal NaOCl Concentration (mg/L) | Fecal Production (%) | | | | |
|---|---|---|---|---|---|---|
| | | Range | $\bar{x}$ | $s/\sqrt{n}$ | n | t-Value |
| A | 0.00 | 60-100 | 83.4 | 1.54 | 41 | |
| B | 1.00 | 20-100 | 79.2 | 1.56 | 40 | |
| C | 0.56 | 48-100 | 80.3 | 2.08 | 40 | |
| D | 0.10 | 64-100 | 82.5 | 1.65 | 39 | |
| E | 0.00 | 4-100 | 64.7** | 3.61 | 38 | -4.76[a] |
| F | 1.00 | 16-92 | 65.5** | 2.61 | 38 | -4.51[b] |
| G | 0.56 | 12-92 | 72.5** | 2.28 | 39 | -2.53[c] |
| H | 0.10 | 12-92 | 66.9** | 3.19 | 39 | 4.34[d] |

[a] [b] t-value calculated from a comparison of treatments A and E.
[c] t-value calculated from a comparison of treatments B and F.
[d] t-value calculated from a comparison of treatments C and G.
t-value calculated from a comparison of treatments D and H.

Gill tissue respiration values are depicted graphically in Figure 1B. Mean gill tissue respiration rates declined throughout the study in both high ($\bar{x}$ = 1280.6 to 1201.5 µl $O_2$/g/h) and low salinity ($\bar{x}$ = 1280.6 to 1201.3 µl $O_2$/g/h) controls in response to declining sea water temperatures (28-20°C) measured in the study. There were no significant differences in respiration rates measured among oysters in comparison of high and low salinity controls.

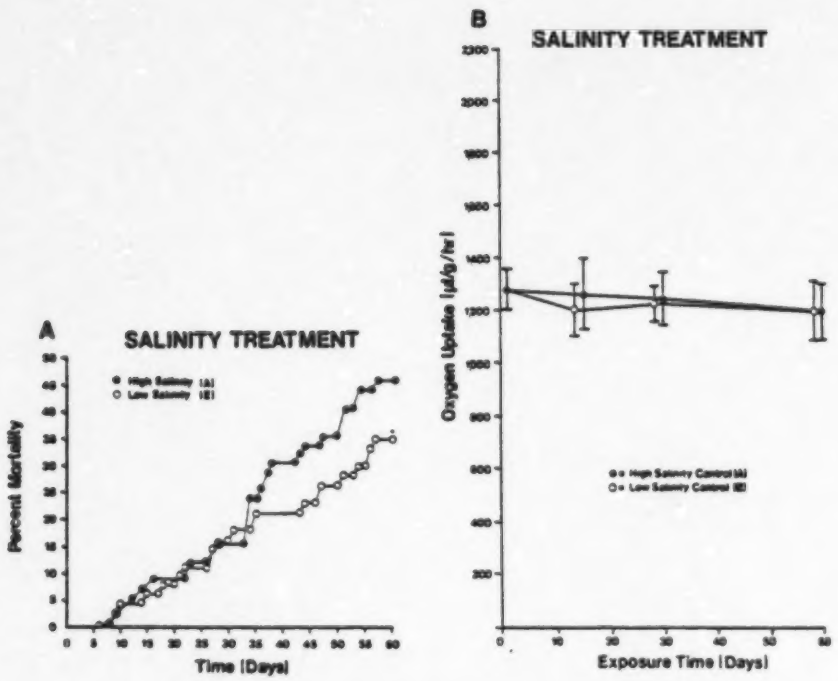


Figure 1A. Mortality data for oysters exposed to high (A) and low (E) salinity treatments. Note that mortality differences between the two treatments were only significant after 30 days of exposure when temperatures fell below 25°C. Asterisk (*) indicates that 60-day mortality results were significantly ($p<0.05$) different. Standard error values were less than ± 4% in both treatments.

1B. Mean gill tissue respiration rates (µl $O_2$/g/h) measured in oysters exposed to high (A) and low (E) salinity treatments. Note the similarity in respiration rates among high and low salinity oysters throughout the entire 60 days of exposure. Bars (I) represent sample standard error.

Hypnospore densities of P. marinus in rectal and labial palp tissues of surviving oysters are listed in Table 3. The incidence of infection was 100% for both high and low salinity control oysters. Hypnospore densities in rectal tissues increased throughout the 60 d of exposure in both high salinity controls ($\bar{x}$ = 66.23 to 670.18 hypnospores per 5-mm field) and low salinity controls ($\bar{x}$ = 75.8 to 1333.3 hypnospores per 5-mm field). Statistical analysis indicated that hypnospore densities were not significantly different between rectal and labial palp tissues in oysters exposed to either high or low salinity. Additionally, hypnospore densities in rectal tissues were not significantly different in comparisons between high and low salinity treatments. Also, hypnospore densities in labial palp tissues were not significantly different between high and low salinity controls.

Weighted indices of infection-intensity calculations for P. marinus in rectal and labial palp tissues are listed in Table 4. Initially, low salinity exposure significantly ($p<0.01$) lowered infection intensities in both rectal and labial palp tissues as hypnospore densities were substantially reduced during the first 15 d of exposure. However, after 30 d of exposure and continuing through 60 d of exposure, infection intensities were practically the same for low and high salinity controls in both rectal and labial palp tissues.

## Chlorination Treatment

Nominal NaOCl and measured CPO concentrations are listed in Table 1 for both high and low salinity exposures. Measured CPO concentration for each selected nominal NaOCl concentration varied significantly in both high and low salinity, chlorinated sea water combinations. Further analysis of these results indicated that measured CPO concentrations for each selected nominal NaOCl concentration were significantly ($p<0.01$-$0.05$) higher (45%-50%) in low salinity, chlorinated sea water exposures (F, G, H) when compared with high salinity, chlorinated sea water exposures (B, C, D). Higher CPO concentrations in the low salinity sea water exposures would be expected due to the lower chlorine demand (produced by the presence of all forms of dissolved and suspended organic matter) exerted by the low salinity sea water (as it was diluted with groundwater which reduced phytoplankton densities by 66%).

Results indicating the effect of chlorination on oyster mortalities associated with infections of P. marinus are

Table 3. Mean rectal and labial palp hypnospore densities in oysters exposed to various chlorination/salinity conditions. Hypnospore densities were not significantly different in comparison of high and low salinity, CPO-exposed oysters or in comparisons of controls. Statistical parameters included calculations of mean ($\bar{x}$), standard error ($s/\sqrt{n}$), sample size (n), and Students t-value.

| Treatment | Exposure Time (days) | Exposure Temperature (°C) | Rectum Hypnospore Density[a] | | | | | Labial Palp Hypnospore Density[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\bar{x}$ | $s/\sqrt{n}$ | n | t-value[1] | t-value[2] | $\bar{x}$ | $s/\sqrt{n}$ | n | t-value[1] | t-value[2] |
| A | 0 | 28 | 77.4 | 66.23 | 14 | | | 75.8 | 64.89 | 15 | | |
| A | 15 | 27 | 61.1 | 34.74 | 14 | | | 37.5 | 25.56 | 15 | | |
| B | 15 | 27 | 2021.4 | 1065.53 | 15 | | +1.84 | 2007.7 | 1068.30 | 15 | | +1.86 |
| C | 15 | 27 | 1585.2 | 939.67 | 15 | | +1.62 | 1495.1 | 916.13 | 15 | | +1.59 |
| D | 15 | 27 | 81.6 | 70.77 | 15 | | +0.26 | 62.5 | 53.88 | 15 | | +0.42 |
| E | 15 | 27 | 3.3 | 1.41 | 15 | -1.66 | | 2.9 | 1.24 | 15 | -1.35 | |
| F | 15 | 27 | 1058.4 | 559.58 | 15 | +0.80 | +1.89 | 1225.2 | 668.95 | 15 | -0.62 | +1.83 |
| G | 15 | 27 | 2122.9 | 1,131.79 | 15 | +0.37 | +1.87 | 1729.9 | 998.62 | 15 | +0.17 | +1.73 |
| H | 15 | 27 | 39.5 | 22.55 | 15 | -0.57 | +1.59 | 32.9 | 23.04 | 15 | -0.51 | +1.30 |
| A | 30 | 24 | 703.3 | 692.84 | 15 | | | 753.9 | 691.70 | 15 | | |
| B | 30 | 24 | 27.7 | 7.41 | 15 | | -0.98 | 29.1 | 15.75 | 15 | | -1.05 |
| C | 30 | 24 | 712.8 | 697.73 | 15 | | +0.01 | 747.8 | 697.41 | 15 | | -0.01 |
| D | 30 | 24 | 771.0 | 713.80 | 15 | +0.70 | | 142.2 | 85.31 | 15 | -0.88 | |
| E | 30 | 24 | 14.1 | 9.00 | 15 | | -0.99 | 10.5 | 6.07 | 15 | | -1.07 |
| F | 30 | 24 | 1009.5 | 726.23 | 15 | +1.35 | +0.23 | 997.7 | 726.04 | 15 | +1.33 | +1.17 |
| G | 30 | 24 | 795.6 | 759.27 | 15 | +0.08 | +0.02 | 799.9 | 76.85 | 15 | +0.05 | +0.84 |
| H | 30 | 24 | 155.3 | 90.69 | 15 | +1.55 | -0.86 | 130.4 | 64.92 | 15 | +1.84 | -0.11 |
| A | 60 | 20 | 765.0 | 670.18 | 15 | | | 752.9 | 658.44 | 15 | | |
| B | 60 | 20 | 1578.1 | 961.99 | 15 | | +0.69 | 1508.2 | 952.28 | 15 | | +0.65 |
| C | 60 | 20 | 546.6 | 538.36 | 15 | | -0.25 | 696.5 | 684.71 | 15 | | -0.06 |
| D | 60 | 20 | 800.1 | 698.15 | 15 | | +0.04 | 809.1 | 714.65 | 15 | | +0.06 |
| E | 60 | 20 | 702.2 | 631.14 | 15 | -0.07 | | 1333.3 | 902.11 | 15 | -0.52 | |
| F | 60 | 20 | 2141.2 | 1100.10 | 15 | +0.39 | | 1792.7 | 945.77 | 15 | +0.21 | +0.35 |
| G | 60 | 20 | 802.9 | 706.70 | 15 | -0.29 | +0.11 | 659.2 | 538.94 | 15 | -0.04 | -0.64 |
| H | 60 | 20 | 746.4 | 724.33 | 15 | -0.05 | +0.05 | 756.1 | 742.73 | 15 | -0.05 | -0.49 |

[a]Hypnospore per 5-mm field.
[1]Comparison of high salinity, CPO treatments with similar low salinity, CPO treatments (B vs. F, C vs. G, and D vs. H) and comparisons of high and low salinity controls (A vs. E).
[2]Comparison of high salinity controls (A) with high salinity, CPO-exposed oysters (B, C, D) and comparisons of low salinity controls (E) with low salinity, CPO-exposed oysters (F, G, H).

Table 4. Mean rectal and labial palps weighted indices of infection intensities (0-7) measured in oysters exposed to various chlorination/salinity conditions. No significant differences in infection intensities were observed in comparisons of high and low salinity, CPO-exposed oysters, but differences between high and low salinity controls and CPO-exposed oysters were observed. Statistical parameters included calculations of mean ($\bar{x}$), standard error ($s/\sqrt{n}$), sample size (n), and Students t-value. Asterisks (*) (**) indicate reported t-values were significant ($p<0.05$ and $p\leq 0.01$, respectively).

| Treatment | Exposure Time (days) | Exposure Temperature (°C) | Rectum Hypnospore Density[a] | | | | | Labial Palp Hypnospore Density[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\bar{x}$ | $s/\sqrt{n}$ | n | t-value[1] | t-value[2] | $\bar{x}$ | $s/\sqrt{n}$ | n | t-value[1] | t-value[2] |
| A | 0 | 28 | 1.73 | 0.36 | 15 | | | 1.60 | 0.38 | 15 | | |
| A | 15 | 27 | 3.60 | 0.30 | 15 | | | 2.13 | 0.35 | 15 | | |
| B | 15 | 27 | 3.00 | 0.59 | 15 | | +0.56 | 2.87 | 0.62 | 15 | | +1.04 |
| C | 15 | 27 | 3.33 | 0.55 | 15 | | +1.07 | 2.80 | 0.56 | 15 | | +1.01 |
| D | 15 | 27 | 1.13 | 0.22 | 15 | -3.22** | | 1.20 | 0.14 | 15 | | |
| E | 15 | 27 | 2.07 | 0.37 | 15 | | -0.97 | 1.87 | 0.35 | 15 | | -0.53 |
| F | 15 | 27 | 3.00 | 0.61 | 15 | 0.00 | +2.80** | 3.13 | 0.61 | 15 | +0.30 | +3.08** |
| G | 15 | 27 | 2.60 | 0.62 | 15 | -0.88 | +2.23* | 2.40 | 0.63 | 15 | -0.47 | +1.86 |
| H | 15 | 27 | 2.33 | 0.39 | 15 | +0.48 | +2.68* | 2.00 | 0.34 | 15 | +0.27 | +2.18* |
| A | 30 | 24 | 2.33 | 0.41 | 15 | | | 2.27 | 0.47 | 15 | | |
| B | 30 | 24 | 2.73 | 0.27 | 15 | | +0.81 | 2.27 | 0.32 | 15 | | 0.00 |
| C | 30 | 24 | 2.47 | 0.44 | 15 | | +0.23 | 2.20 | 0.46 | 15 | | -0.11 |
| D | 30 | 24 | 1.67 | 0.25 | 15 | | -1.23 | 2.20 | 0.46 | 15 | | -1.65 |
| E | 30 | 24 | 2.40 | 0.50 | 15 | +0.11 | | 2.33 | 0.51 | 15 | | |
| F | 30 | 24 | 2.07 | 0.56 | 15 | -1.06 | -0.44 | 2.20 | 0.55 | 15 | -0.11 | -0.17 |
| G | 30 | 24 | 2.00 | 0.47 | 15 | -0.73 | -0.58 | 2.00 | 0.43 | 15 | -0.32 | -0.49 |
| H | 30 | 24 | 2.27 | 0.44 | 15 | +1.19 | -0.20 | 2.20 | 0.46 | 15 | +1.54 | -0.19 |
| A | 60 | 20 | 2.47 | 0.51 | | | | 2.40 | 0.50 | 15 | | |
| B | 60 | 20 | 3.47 | 0.74 | 15 | | +1.11 | 3.60 | 0.48 | 15 | | +1.73 |
| C | 60 | 20 | 2.00 | 0.45 | 15 | | -0.69 | 2.00 | 0.45 | 15 | | -0.59 |
| D | 60 | 20 | 2.73 | 0.49 | 15 | | +0.37 | 2.53 | 0.46 | 15 | | +0.19 |
| E | 60 | 20 | 2.27 | 0.49 | 15 | -0.40 | | 2.40 | 0.55 | 15 | | |
| F | 60 | 20 | 3.40 | 0.55 | 15 | -0.08 | +1.53 | 3.07 | 0.56 | 15 | -0.72 | +0.85 |
| G | 60 | 20 | 2.53 | 0.51 | 15 | -0.78 | +0.37 | 2.60 | 0.49 | 15 | +0.90 | +0.27 |
| H | 60 | 20 | 2.33 | 0.44 | 15 | -0.61 | +0.09 | 2.07 | 0.45 | 15 | -0.71 | -0.46 |

[a]Hypnospore per 5-mm field.

[1]Comparison of high salinity, CPO treatments with similar low salinity, CPO treatments (B vs. F, C vs. G, and D vs. H) and comparisons of high and low salinity controls (A vs. E).

[2]Comparison of high salinity controls (A) with high salinity, CPO-exposed oysters (B, C, D) and comparisons of low salinity controls (E) with low salinity, CPO-exposed oysters (F, G, H).

depicted in Figure 2A. Oyster mortalities were significantly ($p<0.05$) lower in each low salinity, chlorination treatment when compared with equivalent high salinity, chlorination treatments. Mean oyster mortalities averaged 22.2% (ranging from 20 to 25%) in the various low salinity, chlorination exposures (F, G, H) compared to an average mortality rate of 51.1% (ranging from 41.7 to 56.7%) in the various high salinity, chlorination treatments (B, C, D).

Comparisons of mortality rates in the various high salinity, chlorination exposures (B, C, D) with high and low salinity controls (A and E, respectively) indicated the mean mortality rate in the nominal 1.00 mg/L, high salinity treatment (B = 56.7%) was significantly ($p<0.05$) higher than the mean mortality rates recorded in high and low salinity control oysters (A = 46.7% and E = 35%, respectively). The mean mortality rates in the nominal 0.56 mg/L and 0.10 mg/L, high salinity treatments (C = 45% and D = 41.7%, respectively) were slightly lower but not significantly different from the mortality rate for high salinity, control oysters (A = 46.7%). The mean mortality rates in the nominal 0.56 mg/L and 0.10 mg/L, high-salinity treatments (C = 45% and D = 41.7%, respectively) were significantly ($p<0.05$) higher than the mortality rate for low salinity, control oysters (E = 35%).

Comparisons of mortality rates in the various low salinity, chlorination exposures (F, G, H) with high and low salinity controls (A and E, respectively) indicated that the mean mortality rates were significantly ($p<0.05$) lower in each of the low salinity, chlorination treatments (F = 25%, G = 21.7%, and H = 20%) when compared to either the high salinity (A = 46.7%) or low salinity (E = 35%) controls.

Mortality was lowest (averaging 20%) in the 0.10 mg/L nominal NaOCl concentration, low salinity treatment (H). This represented a 57% reduction in the mortality rate measured in high salinity control oysters (A) and a 43% reduction in the mortality for low salinity control oysters (E).

Measurements of fecal production or biodeposition rates are listed in Table 2. Fecal production rates were significantly ($p<0.01$) lower in each low salinity, chlorination exposure when compared with equivalent high salinity, chlorination exposures. Mean fecal production rates averaged 68.3% (ranging from 65.6% to 72.5%) in the various low salinity, chlorination exposures (F, G, H) compared to an average rate of 80.7% (ranging from 79.2 to 82.5%) in the different high salinity, chlorination exposures (B, C, D). These results indicated that oyster fecal production in

chlorinated, high salinity sea water was significantly ($p<0.05$-$0.01$) higher than in chlorinated, low salinity sea water. Further comparisons with high and low salinity, control oysters indicated that reduced salinity (or dilution), rather than chlorination, had a more profound effect on fecal production, probably related to the lower phytoplankton densities (food availability) in the low salinity, chlorination exposures.

Gill tissue respiration measurements for oysters exposed to various CPO concentration/salinity exposure regimes are depicted in Figure 2B. Gill tissue respiration rates in both high and low salinity, chlorination exposures initially increased from day 0 to day 15, then gradually declined from day 15 to day 60. Gill tissue respiration rates in high salinity, CPO-exposed oysters (B, C, D) generally were not significantly different from values measured in the low salinity, CPO exposures (F, G, H). The only exception to this trend occurred at days 30 and 60 in the 1.00 mg/L nominal NaOCl concentration, high salinity treatment values (B = 1568.7 and 1598.3 µl $O_2$/g/h, respectively) which were significantly ($p<0.01$) higher than corresponding low salinity, chlorination treatment values (F = 1110.5 and 1179.2 µl $O_2$/g/h, respectively) and high (A) and low (E) salinity control values.

Comparisons of high (A) and low salinity (E) controls with the other high salinity, chlorination exposures (C, D) indicated that gill tissue respiration rates generally were not significantly different between controls and high salinity, CPO exposures. Similar comparisons of different low salinity, CPO-exposed oysters (F, G, H) with high (A) and low (E) salinity controls indicated that generally gill tissue respiration rates of controls were not significantly different from low salinity, CPO exposures.

Hypnospore densities of P. marinus in rectal and labial palp tissues are listed in Table 3. The incidence of infection was 100% for both high salinity (B, C, D) and low salinity (F, G, H), CPO-exposed oysters. Hypnospore densities in rectal and labial palp tissues were variable but generally increased in both rectum (in 83% of the samples) and labial palp tissues (in 72% of the samples) throughout the 60 d of exposure in both high and low salinity, CPO-exposed oysters. Statistical analysis of high salinity (B, C, D) and low salinity (F, G, H), CPO-exposed oysters indicated that there were no significant differences in comparisons of rectal tissue hypnospore densities. Similarly, there were no significant differences in hypnospore

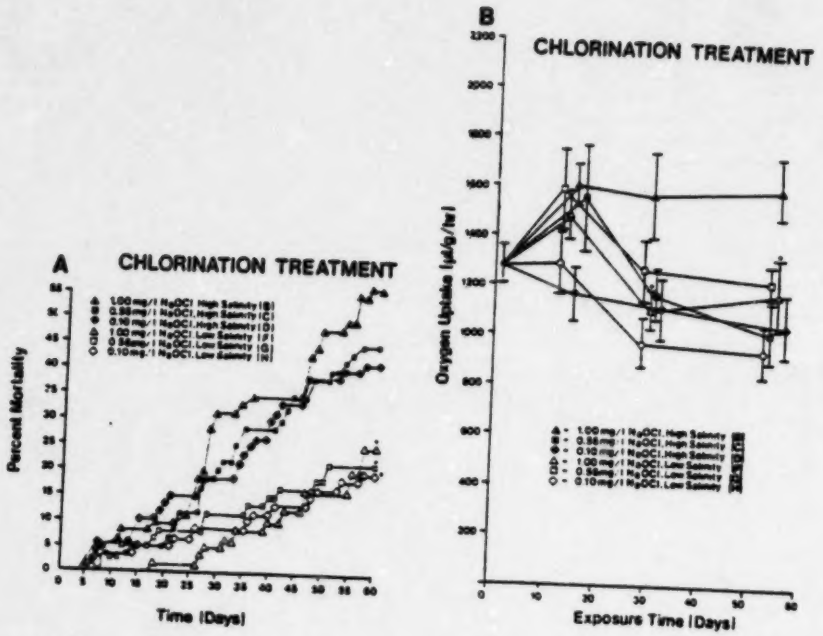


Figure 2A. Mortality data for oysters exposed to various high salinity (B, C, D) and low salinity (F, G, H), chlorination treatments. Note the significantly ($p \leq 0.05$) higher mortality in the high salinity, CPO-exposed oysters. Asterisk (*) indicates that 60-day mortality results were significantly ($p \leq 0.05$) different. Standard error values were less than ± 4% in all treatments throughout the 60 days of exposure.

2B. Mean gill tissue respiration rates (µl $O_2$/g/h) measured in adult oysters exposed to various high salinity (B, C, D) and low salinity (F, G, H), chlorination treatments for 60 days. Gill tissue respiration rates generally declined throughout the 60 days of exposure as a result of declining sea water temperatures. Note the similarity in gill tissue respiration rates between high and low salinity, CPO-exposed oysters with the exception of Treatment B at exposure days 30 and 60. Asterisk (*) indicates that 30- and 60-day gill tissue respiration rates in Treatment B were significantly ($p \leq 0.01$) higher than in Treatment F. Bars (I) represent sample standard error.

densities in labial palp tissue, between high and low salinity, chlorination treatments. Comparisons within each salinity/chlorination treatment indicated that rectal hypnospore densities were generally greater than labial palp tissue densities. Additional comparisons of high salinity (B, C, D) and low salinity (F, G, H), CPO-exposed oysters with high (A) and low salinity (E) controls indicated there were no significant differences between controls and the various chlorination treatments in hypnospore densities of rectal and labial palp tissues.

The weighted index of infection-intensity calculations for P. marinus in rectal and labial palp tissues are listed in Table 4. Infection intensity in both rectal and labial palp tissues generally increased (in 90% of the samples) throughout the 60 d of exposure of high salinity (B, C, D) and low salinity (F, G, H), CPO-exposed oysters. The general trend observed for most salinity/chlorination treatments was an initial increase in infection intensity (for both rectum and labial palps) from day 0 to day 15, followed by a slight decrease from day 15 to day 30, with a final increase being observed from day 31 to day 60.

Statistical analysis of high salinity (B, C, D) and low salinity (F, G, H), CPO-exposed oysters indicated there were no significant differences in comparison of weighted index of infection intensities among rectal tissues. Similarly, there were no significant differences in comparisons of the weighted index of infection intensities among labial palp tissues.

Comparisons of high salinity (B, C, D) and low salinity (F, G, H), CPO-exposed oysters with high (A) and low salinity (E) controls showed no significant differences in rectal infection intensities between controls and the various salinity/chlorination treatments. The same results were obtained for labial palp tissue.

## DISCUSSION

Results from this study have generally indicated the significant interactive effects of salinity and CPO exposure in affecting oyster mortalities resulting from P. marinus infection.

Comparison of control oysters in high and low salinity exposures generally indicated that low salinity conditions resulted in exposure of oysters to water which was reduced in both salt content (major ions) and phytoplankton density (as food availability was reduced by 66%). Biodeposition

rates (or feeding rates) were significantly reduced in low salinity exposed oysters, probably related to less food availability. Galtsoff (1964) reported similar reduced feeding rates in oysters exposed to low salinity. Exposure to low salinity conditions and accompanying reduced fecal production rates did not significantly affect oyster physiology as measurements of condition index, gonadal index, and gill tissue respiration rates were not significantly different (and were generally identical) throughout the 60 d of exposure (Scott, unpubl. data).

Analysis of mortality data from high and low salinity control conditions indicated that low salinity exposure significantly increased oyster survivability of P. marinus infections by 25%. This significant increase in survival occurred in low salinity control oysters even though there were generally no statistically significant differences in the incidence of infection, hypnospore density, and weighted index of infection intensity in comparisons of high and low salinity control oysters. Analysis of the weighted index of infection-intensity data suggested that low salinity exposure initially decreased infection intensity (at day 15) but that by days 30 and 60, infection intensities were equal for both high and low salinities. Earlier studies (Mackin & Wray, 1952; Mackin, 1956) have indicated a similar delay in hypnospore development from low salinity exposure.

While infection-intensity differences in comparisons of high and low salinity control oysters were only slight, actual differences may have been obscured by the higher mortality rate in high salinity control oysters, since the most heavily infected oysters were presumably dying first. This can be presumed since reported measurements were not made of gapers but rather of living specimens. The death of the most diseased individuals would result in selection of more resistant and/or less infected oysters which would tend to bias infection intensities downward to much lower levels than if all heavily diseased or dead individuals were included. Increased mortality among various treatments was therefore considered indicative of higher infection rates as all autopsied dead oysters had infection intensities of 7 (Scott, unpubl. data).

The mode of action for low salinity exposure in significantly reducing oyster mortalities has been reviewed by several authors (Ray, 1954; Andrews & Hewatt, 1957) who generally concluded that low salinity exposure must enhance survival due to possible reductions in the number of waterborne zoospores which would tend to limit and reduce both

the incidence and intensity of infection. However, the fact that all oysters in this study were infected with P. marinus at day 0 and since the infection spreads only from the initial site of infection suggests that differences in survival were not related to differences in zoospore density in sea water, but rather to physiological differences in oysters exposed to different salinity.

Results from chlorination (CPO) exposure treatment indicated that the combination of low salinity and CPO exposure significantly ($p<0.05$) reduced oyster mortalities by 47-57% below high salinity control levels at all chlorine concentrations tested (0.10-1.00 mg/L nominal NaOCl concentrations; measured CPO concentrations of 0.02-0.11 mg/L). The combination of high salinity and CPO exposure increased mortality by 21% above high salinity control levels at high chlorination doses (1.00 mg/L nominal NaOCl concentration; measured CPO concentration of 0.06 mg/L), while in lower CPO concentrations mortality was equal to or was slightly lower than high salinity control mortality rates. Earlier studies (Scott, 1979; Scott & Vernberg, 1979) reported similar increased mortality 14% above control values in oysters exposed to a 1.00 mg/L nominal NaOCl concentration in high salinity waters during the same time period of the year.

Additional comparisons also indicated that the combination of low salinity and CPO exposure significantly reduced oyster mortality below low salinity control values.

Fecal production measurements indicated increased feeding rates in high salinity, CPO-exposed oysters (79.2-82.5%) when compared to low salinity, CPO-exposed oysters (65.5-72.5%). Fecal production rates in high and low salinity, CPO-exposed oysters were not significantly different from rates for each respective high and low salinity control. Decreased fecal production was considered attributable to low salinity effects rather than CPO exposure. These results differ significantly from earlier results obtained by Scott (1979), Scott and Middaugh (1978), and Scott and Vernberg (1979), who reported that CPO exposure caused significant reductions in fecal production rates at low chlorine concentrations (nominal 1.00 mg/L). However, these earlier studies were continuous-exposure, flow-through experiments in comparison to the intermittent (8-12 h of CPO exposure/d) conditions used in this study.

Comparisons of gill tissue respiration rates in high and low salinity, CPO-exposed oysters generally indicated that respiration rates were slightly lower in low salinity, CPO-exposed oysters. Gill respiration rates were only

significantly ($p<0.01$) increased in oysters in the 1.00 mg/L nominal NaOCl concentration, high salinity exposure. Scott (1979), Scott and Vernberg (1979), and Scott et al. (1980) have reported similar increased gill tissue respiration rates in oysters chronically (60 d) exposed to 1.00 mg/L nominal NaOCl concentration under similar exposure conditions, which resulted from increased anaerobic metabolism by oysters to survive CPO exposure.

Results from this study suggested that CPO exposure at similar chlorine concentrations (1.00 mg/L nominal NaOCl) increased gill tissue respiration rates, but only under high salinity conditions. Higher gill tissue respiration rates in these oysters resulted from increased reliance upon gonadal glycogen reserves to survive CPO exposure under high salinity conditions. Absence of these effects in lower CPO concentrations under high salinity exposure and in low salinity, CPO exposures may imply that CPO exposure must interact with factors representative of normal high salinity exposure conditions per se, such as increased P. marinus infection intensity (indicated by higher mortality), to cause increased stress in these oysters. Such an interaction would result in a more rapid depletion of stored gonadal glycogen reserves due to increased metabolic utilization of glycogen by the oyster to survive CPO exposure and by the parasite as the infection intensifies and spreads. There was a rapid decline of gonadal indices in high salinity, CPO-exposed oysters in this study which may be indicative of this effect (Scott, unpubl. data). The rapid depletion of gonadal glycogen stores resulting from this combined host/parasite effect would probably result in earlier utilization of protein stores (amino acids) as glycogen levels declined. This would signify utilization of different metabolic pathways both by the host (oyster) and parasite and may result in an ultimate buildup of different secondary metabolites which may adversely affect the oyster. This increased mortality rate observed in 1.00 mg/L nominal NaOCl concentration, high salinity exposed oysters was probably indicative of this combined toxicant/host/parasite effect.

Consideration must be given to physiological and biochemical differences between high and low salinity, control oysters. Oysters are euryhaline organisms surviving wide ranges of salinity as low as 5-7°/$_{oo}$ (Galtsoff, 1964; Vernberg & Vernberg, 1972). Additionally, oysters are considered osmoconformers which prefer salinities of 11-30°/$_{oo}$ (Galtsoff, 1964; Dupuy et al., 1977). Osmoregulation in low salinity sea water appears to result from volume regulation,

the result of intracellular solute loss (Pierce, 1971). The source of these intracellular solutes has been reported to be in the intracellular, free amino acid pool. Decreased levels of taurine, alanine, glycine, and proline have been reported in intertidal bivalve species, while reduced levels of taurine, alanine, and glycine have been reported in subtidal bivalve species (Pierce, 1971). Under low salinity exposure, free amino acid levels may decline by as much as 84%, particularly ninhydrin positive (i.e., glycine and alanine) substances (Lange, 1970; Vikar & Webb, 1970). Low salinity exposure would significantly reduce the amount of intracellular, free amino acids in oysters during this study.

This reduction of free amino acid levels in low salinity control oysters may, perhaps, explain the high survival observed under low salinity exposure. Low salinity oysters may have increased survival due to the reduced availability of protein or amino acids as food substrate for P. marinus, particularly as the infection intensifies to heavy intensities.

Additional consideration must be given to results from Soniat and Koenig (1982) who found that free amino acid levels in oysters declined with increasing levels of P. marinus infection. This suggests increased utilization of free amino acids by both the host (oyster) and parasite during periods of infection. Soniat and Koenig (1982) also reported increased levels of taurine and aspartic acid with increased infection intensity, although total free amino acid levels declined with increasing infection intensity. Similarly, Lange (1963) reported increased levels of free amino acids with increased salinity in Mytilus edulis, with taurine having the most dramatic increase with increased salinity. Giles (1972) reported a 55% decrease in taurine and an 82% decline in aspartic acid levels in Mytilus edulis exposed to low salinity sea water. When these findings are considered, our data may suggest that the higher rates of survival measured in low salinity control and low salinity, CPO-exposed oysters may have resulted from decreased levels of taurine and aspartic acid resulting from osmoregulation at low salinity conditions.

Exposure of oysters to low salinity and low salinity, CPO conditions probably resulted in oysters which had lowered levels of both glycogen and free amino acids. This would initially tend to retard secondary spread and intensification of the disease. The higher mortality rate observed in high salinity controls and high salinity, CPO-exposed

oysters would suggest that the lowered levels of free amino acids in low salinity control and low salinity, CPO-exposed oysters offered some degree of protection to the oyster by preventing the disease from becoming toxic. What is suggested by these data is that the lowered free amino acid levels in low salinity control oysters and low salinity, CPO-exposed oysters may have resulted in less available nitrogen-containing molecules for P. marinus to utilize. Further research in this area is needed to better understand the exact mechanism of host-parasite interactions to chlorine as well as other environmental pollutants.

## LITERATURE CITED

APHA (American Public Health Association). 1971. Standard Methods for Examination of Water and Waste Water, 14th Ed. American Water Works Association, Water Pollution Control Federation, New York. 874 pp.

Andrews, J.D. and W.G. Hewatt, 1957. Temperature control experiments on the fungus disease, Dermocystidium marinum, of oysters. Virginia Fisheries Laboratory 62: 129-133.

Block, R.N., D.T. Burton, S.R. Gullans, and L.B. Richardson. 1977. Respiratory and osmoregulatory responses of white perch (Morone americana) exposed to chlorine and ozone in estuarine waters. pp. 351-360. In: Water Chlorination: Environmental Impact and Health Effects, Vol. 2. R.L. Jolley, H. Gorchev, and D.N. Hamilton, Jr. (eds.). Ann Arbor Press, MI.

Couch, J.A. 1978. Diseases, parasites, and toxic responses of commercial Penaeid shrimps of the Gulf of Mexico and south Atlantic coasts of North America. Fish. Bull. 76(1): 1-44.

Crecelius, E.A., G. Roesijadi, and T.O. Thatcher. 1978. Correspondence. Environ. Sci. Technol. 12: 1088.

Dupuy, J.L., N.T. Windsor, and C.E. Sutton. 1977. Manual for Design and Operation of an Oyster Seed Hatchery for the American Oyster, Crassostrea virginica. Appl. Mar. Sci. and Ocean Eng., Virginia Institute of Marine Science, Special Report No. 142. Gloucester Point, VA. 104 pp.

EPA (Environmental Protection Agency). 1974. Handbook of Analytical Control in Water and Waste Water Laboratories. Anal. Qual. Control. Lab., Cincinnati, OH. 45 pp.

Farley, C.A. 1965. Acid-fast staining of haplosporidian spores in relation to oyster pathology. J. Invert. Pathol. 7(2): 144-147.

Galtsoff, P.S. 1964. The American Oyster, Crassostrea virginica (Gmelin). U.S. Fish and Wild. Serv., Fish. Bull. 64: 1-480.

Giles, R. 1972. Osmoregulation in three molluscs: Acanthochitona discrepans (Brown), Glycymeris glycymeris (L), and Mytilus edulis (L). Biol. Bull. 142: 25-35.

Hall, L.W., Jr., G.R. Helz, and D.T. Burton. 1981. Power Plant Chlorination: A Biological and Chemical Assessment. Ann Arbor Science Publishers, Ann Arbor, MI. 237 pp.

Lange, R. 1963. The osmotic function of free amino acids and taurine in the mussel, Mytilus edulis. Comp. Biochem. Physiol. 10: 173-179.

Lange, R. 1970. Isosmotic intracellular regulation and euryhalinity in marine bivalves. J. Exp. Mar. Biol. Ecol. 5: 170-179.

Mackin, J.G. 1956. Dermosystidium marinum and salinity. Proc. Natl. Shellfish Assoc. 46: 116-128.

Mackin, J.G. 1962. Oyster disease caused by Dermocystidium marinum and other microorganisms in Louisiana. Publ. Inst. Mar. Sci. 7: 132-229.

Mackin, J.G. and D.A. Wray. 1952. Report on the Second Study of Mortality of Oysters in Barataria Bay, Louisiana, and Adjacent Areas. Texas A & M Res. Proj. No. 9, College Station, TX. 40 pp.

Perkins, F.P. 1966. Life history studies of Dermocystidium marinum, an oyster pathogen. Ph.D. Dissertation. Florida State Univ., Tallahassee. Dissertation Abstracts, 66-9078, 272 pp.

Pierce, S.K., Jr. 1971. A source of volume regulation in marine mussels. Comp. Biochem. Physiol. 38A: 619-635.

Quick, J.A. and J.G. Mackin. 1971. Oyster parasitism by Labyrinthomyxa marina in Florida. Florida Department Natural Resources Marine Laboratory, Professional Paper Series 13. 55 pp.

Ray, S.M. 1952. A culture technique for the diagnosis of infections with Dermocystidium marinum (Mackin, Owen, and Collier) in oysters. Science 116(3014): 360-361.

Ray, S.M. 1953. Studies on the occurrence of Dermocystidium marinum in young oysters. Proc. Natl. Shellfish Assoc. 43: 80-88.

Ray, S.M. 1954. Biological Studies of Dermocystidium marinum. a Fungus Parasite of Oysters. Rice Inst.

Pam., Spec. Issue (Nov. 1954), Monographs in Biology. 114 pp.

Ray, S.M. 1966. A review of the culture method for detecting Dermocystidium marinum, with suggested modifications and precautions. Proc. Natl. Shellfish Assoc. 54: 55-69.

Ray, S.M., J.G. Mackin, and J.L. Boswell. 1953. Quantitative measurement of the effect on oysters of the disease caused by Dermocystidium marinum. Bull. Mar. Sci. Gulf and Carib. 3: 6-33.

Roberts, M.H., Jr. and B.B. Casey. In press. Depression of larval growth and metamorphosis of oysters (Crassostrea virginica) exposed to chlorinated sewage. In: Water Chlorination: Environmental Impact and Health Effects, Vol. 5. R.L. Jolley, W.A. Brungs, J.A. Cotruro, R.B. Cummings, J.S. Mattice, and V.A. Jacobs (eds.). Ann Arbor Press, Ann Arbor, MI.

Rosenberg, W.H., J.C. Rhoderick, R.M. Block, V.S. Kennedy, S.R. Gullans, S.M. Vreenegoor, A. Rosenkranz, and C. Collette. 1980. Effects of the chlorine produced oxidants on survival of larvae of the oyster, Crassostrea virginica. Mar. Ecol. Prog. Ser. 3: 91-96.

Scott, G.I. 1979. The effects of seasonal chronic chlorination on the growth, survival, and physiology of the American oyster, Crassostrea virginica (Gmelin). Ph.D. Dissertation. Univ. of South Carolina, Columbia. 169 pp.

Scott, G.I. and D.P. Middaugh. 1978. Seasonal chronic toxicity of chlorination to the American oyster, Crassostrea virginica. pp. 311-328. In: Water Chlorination: Environmental Impact and Health Effects, Vol. 2. R.L. Jolley, H. Gorcher, and D.H. Hamilton, Jr. (eds.). Ann Arbor Press, Ann Arbor, MI.

Scott, G.I. and W.B. Vernberg. 1979. Seasonal effects of chlorine-produced oxidants on the growth, survival, and physiology of the American oyster, Crassostrea virginica (Gmelin). pp. 415-535. In: Marine Pollution: Functional Responses. W.B. Vernberg, F.P. Thurberg, A. Calabrese, and F.J. Vernberg (eds.). Academic Press, New York.

Scott, G.I., D.P. Middaugh, A.M. Crane, N.H. McGlothlin, and N. Watabe. 1980. Physiological effects of chlorine-produced oxidants and uptake of chlorination by-products in the American oyster, Crassostrea virginica (Gmelin). pp. 501-516. In: Water Chlorination: Environmental Impact and Health Effects, Vol. 3.

R.L. Jolley, W.A. Brungs, and R.B. Cumming (eds.). Ann Arbor Press, Ann Arbor, MI.

Scott, G.I. 1983. Physiological effects of chlorine-produced oxidants, dechlorinated effluents, and trihalomethanes on marine invertebrates. pp. 827-842. In: Water Chlorination: Environmental Impact and Health Effects. R.L. Jolley, et al. (eds.). Ann Arbor Press, Ann Arbor, MI.

Sinderman, C.J. 1978. Pollution-associated diseases and abnormalities of fish and shellfish: A review. Fish. Bull. 76(4): 717-749.

Soniat, T.M. and M.L. Koenig. 1982. The effects of parasitism by Perkinsus marinus on the free amino acid composition of Crassostrea virginica mantle tissue. J. Shellfish Res. 2(1): 25-28.

Vernberg, F.J. and W.B. Vernberg. 1972. Environmental Physiology of Marine Animals. Springer-Verlag Press, New York. 346 pp.

Vikar, R.A. and K.L. Webb. 1970. Amino acids in the clam, Mya. Comp. Biochem. Physiol. 32: 775-783.

White, G.C. 1972. Handbook of Chlorination. Van Nostrand Rheinhold Co., New York. 744 pp.